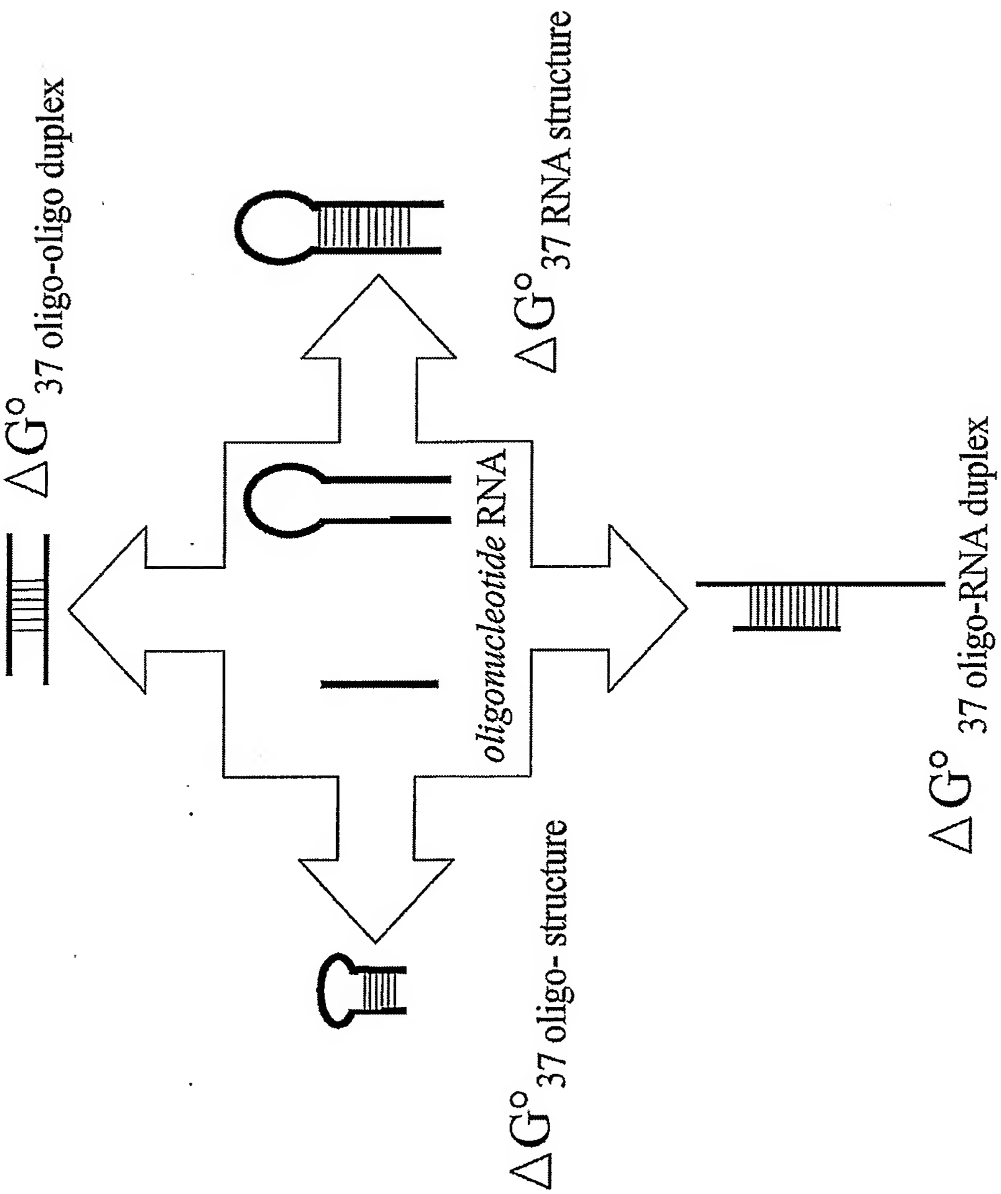

ΔG°_{37} oligo-oligo duplex
ΔG°_{37} RNA structure
oligonucleotide RNA
ΔG°_{37} oligo-RNA duplex
ΔG°_{37} oligo- structure

Figure 1

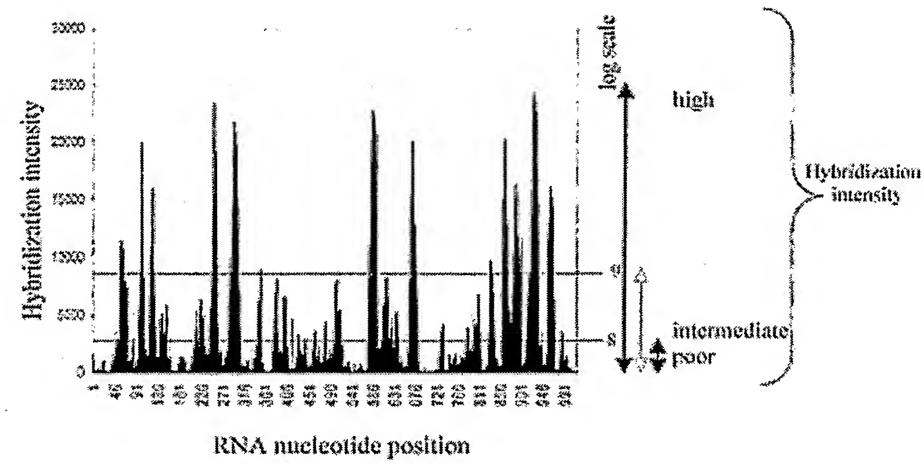

Hybridization intensity
RNA nucleotide position
log scale
high
intermediate
poor
Hybridization intensity

Figure 2

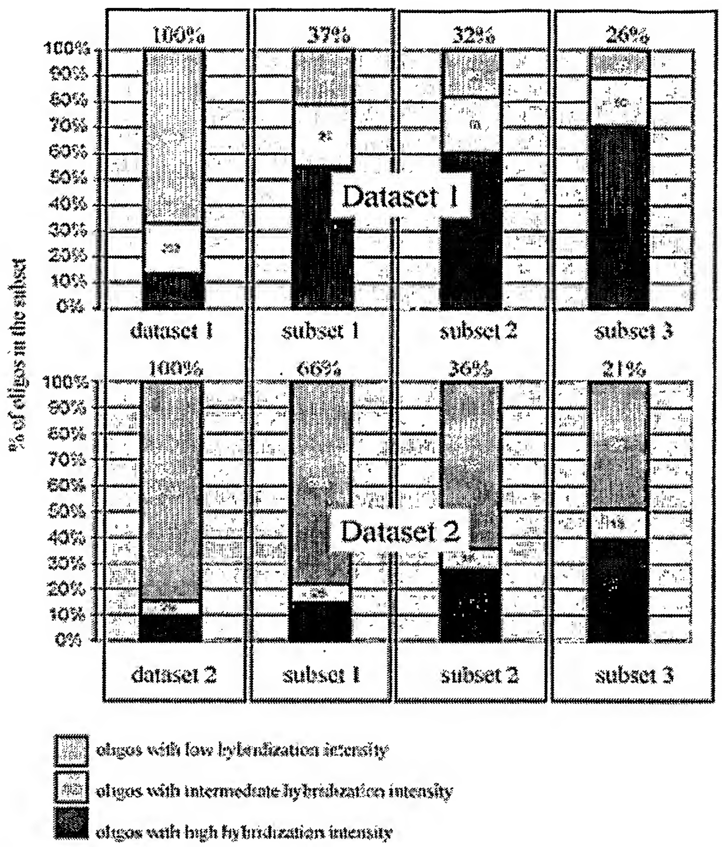
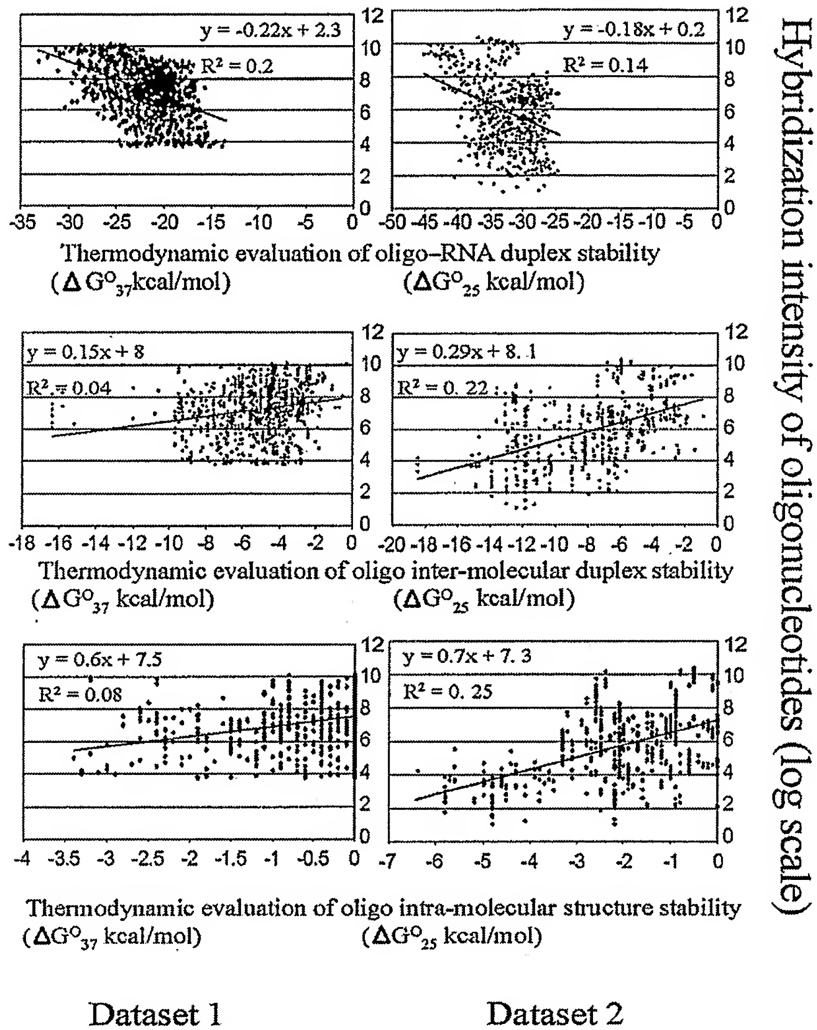

100%
37%
32%
26%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Dataset 1
dataset 1
subset 1
subset 2
subset 3
% of oligos in the subset
100%
66%
36%
21%
Dataset 2
dataset 2
subset 1
subset 2
subset 3
oligos with low hybridization intensity
oligos with intermediate hybridization intensity
oligos with high hybridization intensity

Figure 3



y = -0.22x + 2.3
R² = 0.2
y = -0.18x + 0.2
R² = 0.14
Thermodynamic evaluation of oligo-RNA duplex stability
(ΔG°37 kcal/mol)
(ΔG°25 kcal/mol)
y = 0.15x + 8
R² = 0.04
y = 0.29x + 8.1
R² = 0.22
Thermodynamic evaluation of oligo inter-molecular duplex stability
(ΔG°37 kcal/mol)
(ΔG°25 kcal/mol)
y = 0.6x + 7.5
R² = 0.08
y = 0.7x + 7.3
R² = 0.25
Thermodynamic evaluation of oligo intra-molecular structure stability
(ΔG°37 kcal/mol)
(ΔG°25 kcal/mol)
Hybridization intensity of oligonucleotides (log scale)
Dataset 1
Dataset 2

Figure 4

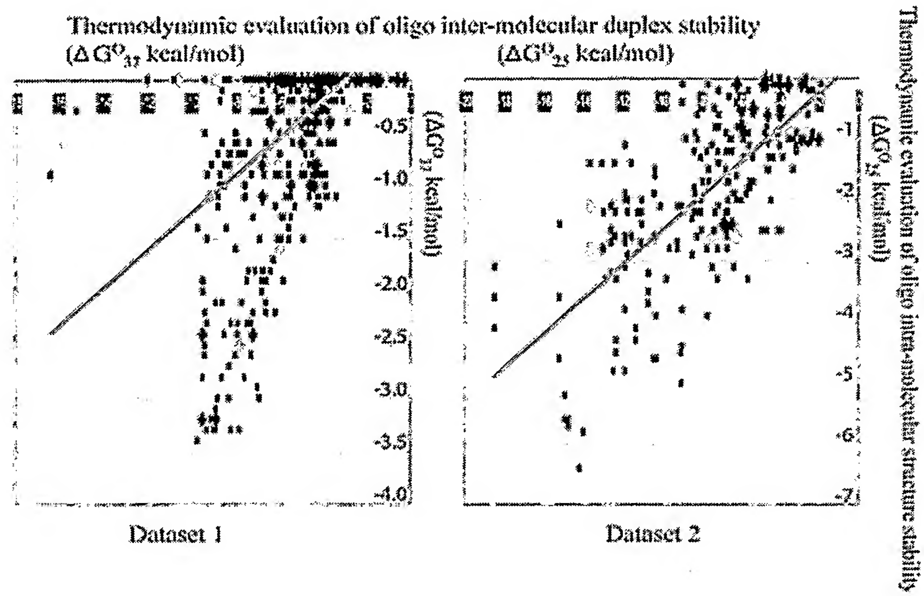

Thermodynamic evaluation of oligo inter-molecular duplex stability
(ΔG°37 kcal/mol)
(ΔG°25 kcal/mol)
(ΔG°37 kcal/mol)
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
Dataset 1
Dataset 2
Thermodynamic evaluation of oligo intra-molecular structure stability
-1
-2
-3
-4
-5
-6
-7

Figure 5

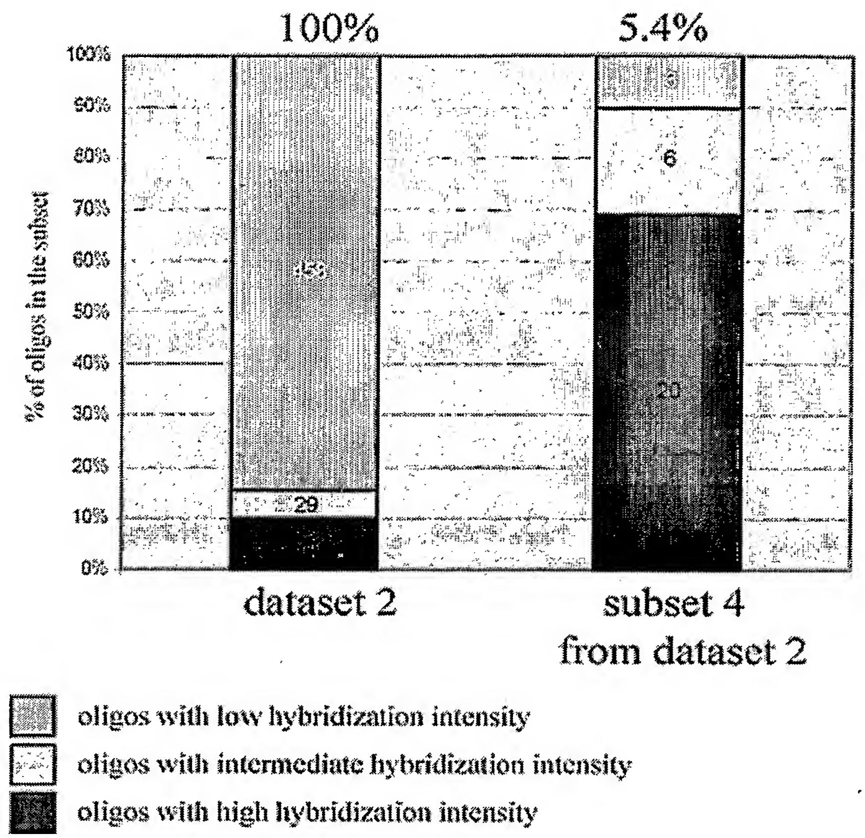

100%
5.4%
% of oligos in the subset
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
29
6
20
dataset 2
subset 4
from dataset 2
oligos with low hybridization intensity
oligos with intermediate hybridization intensity
oligos with high hybridization intensity

Figure 6

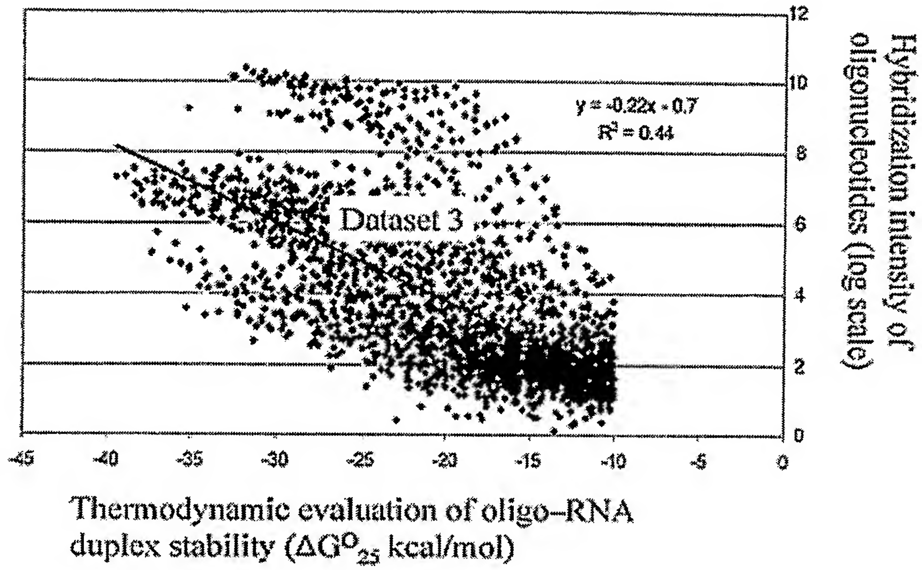

Hybridization intensity of oligonucleotides (log scale)
y = -0.22x - 0.7
R² = 0.44
Dataset 3
12
10
8
6
4
2
0
-45
-40
-35
-30
-25
-20
-15
-10
-5
0
Thermodynamic evaluation of oligo–RNA duplex stability (ΔGO25 kcal/mol)

Figure 7

Selection of the oligo-probe candidate with minimal self-structure. Calculation of delta G duplex between this oligo-probe candidate and its target .

Step 1

Prediction of hybridization intensity value for this-oligo probe using the equation in Figure 7. This value will predict specific hybridization of oligo-probe with its target.

Step 2

Calculation of values of delta G duplex between this oligo-probe and every possible fragment of similar length in genomic DNA.
Selection of the set of duplexes with delta G < -10kcal/mol.

Step 3.

Calculation of intensity of hybridization using the equation from Figure.7 for all duplexes in the set produced in step 3 and calculation of the sum of these predicted intensities of hybridization. This sum will predict the non-specific hybridization intensity of oligo-probe with genomic DNA.

Step 5

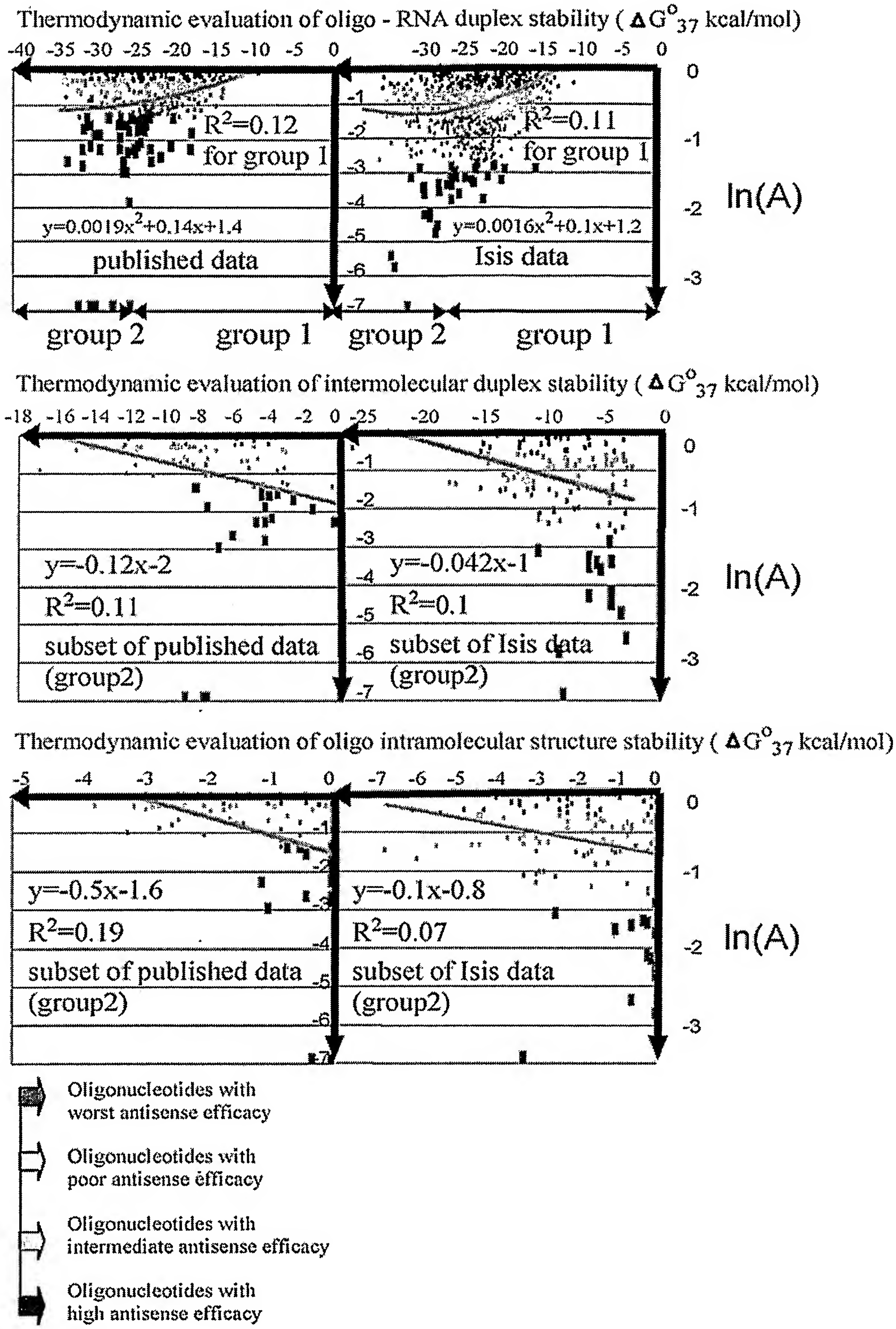
Comparison of value of predicted specific hybridization intensity produced in step (2) and non specific hybridization intensity from step (4).

Step 5

If value of predicted specific hybridization is higher than value of predicted non-specific hybridization, the oligo-probe is a good candidate with poor cross-hybridization potential, otherwise the oligo-probe is a bad candidate.

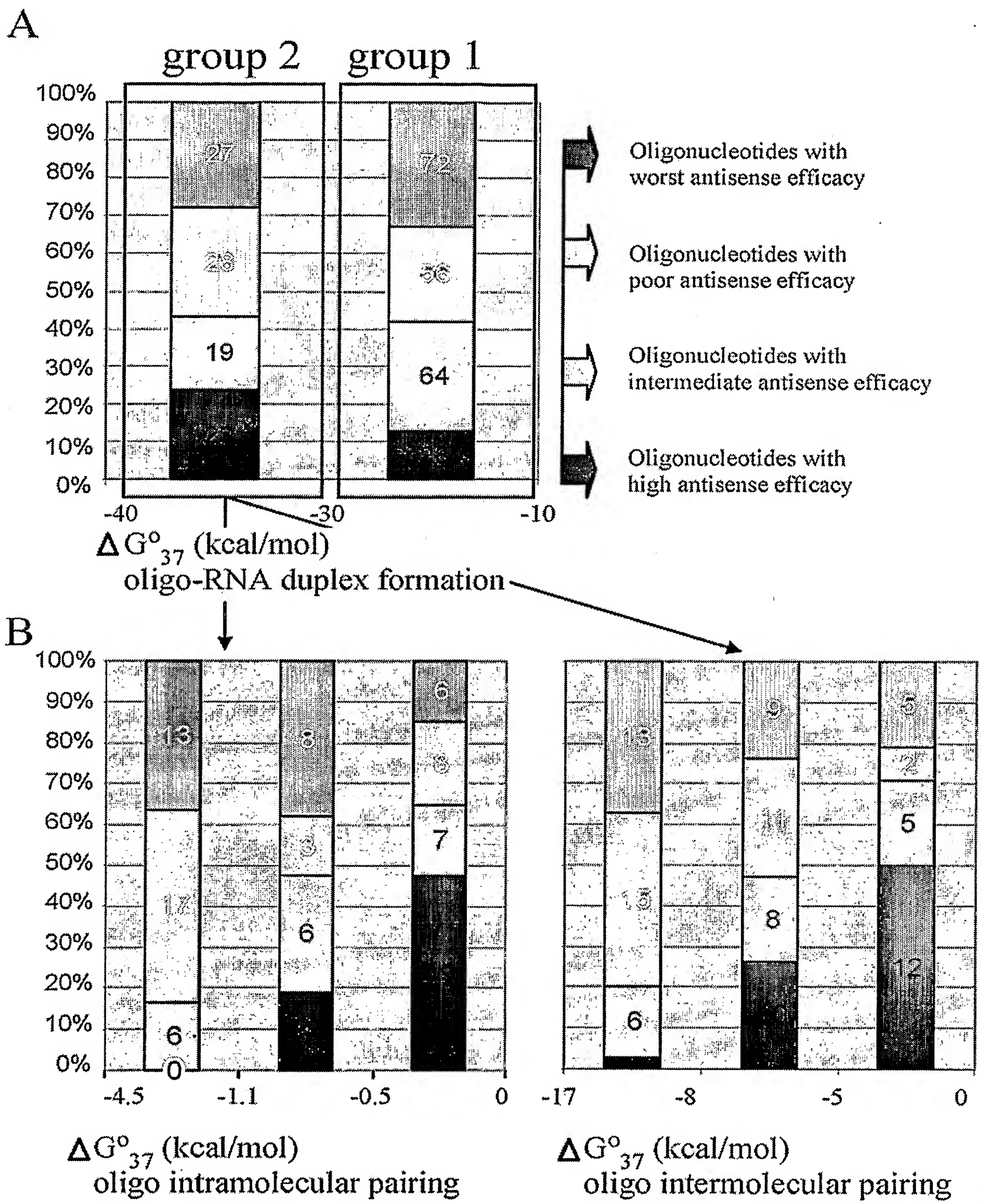
Step 6

Figure 8



Thermodynamic evaluation of oligo - RNA duplex stability (ΔG°37 kcal/mol)
R2=0.12
for group 1
y=0.0019x2+0.14x+1.4
published data
R2=0.11
for group 1
y=0.0016x2+0.1x+1.2
Isis data
group 2
group 1
group 2
group 1
ln(A)
Thermodynamic evaluation of intermolecular duplex stability (ΔG°37 kcal/mol)
y=-0.12x-2
R2=0.11
subset of published data
(group2)
y=-0.042x-1
R2=0.1
subset of Isis data
(group2)
ln(A)
Thermodynamic evaluation of oligo intramolecular structure stability (ΔG°37 kcal/mol)
y=-0.5x-1.6
R2=0.19
subset of published data
(group2)
y=-0.1x-0.8
R2=0.07
subset of Isis data
(group2)
ln(A)
Oligonucleotides with worst antisense efficacy
Oligonucleotides with poor antisense efficacy
Oligonucleotides with intermediate antisense efficacy
Oligonucleotides with high antisense efficacy

Figure 9


A
group 2
group 1
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
27
28
19
72
56
64
Oligonucleotides with worst antisense efficacy
Oligonucleotides with poor antisense efficacy
Oligonucleotides with intermediate antisense efficacy
Oligonucleotides with high antisense efficacy
-40
-30
-10
ΔG°37 (kcal/mol)
oligo-RNA duplex formation
B
13
17
6
0
8
3
6
6
8
7
13
13
6
9
11
8
5
2
5
12
-4.5
-1.1
-0.5
0
-17
-8
-5
0
ΔG°37 (kcal/mol)
oligo intramolecular pairing
ΔG°37 (kcal/mol)
oligo intermolecular pairing

F

Figure 10

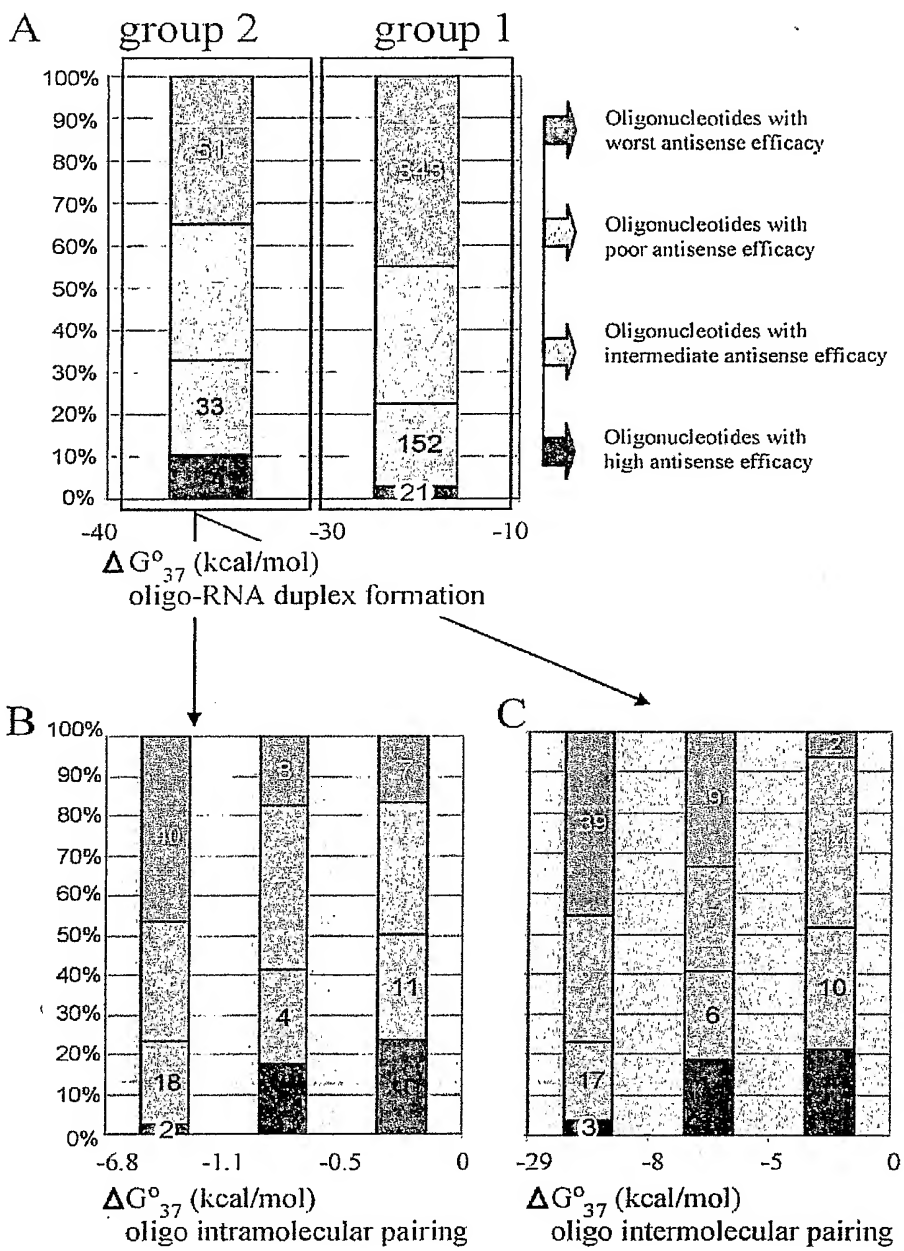
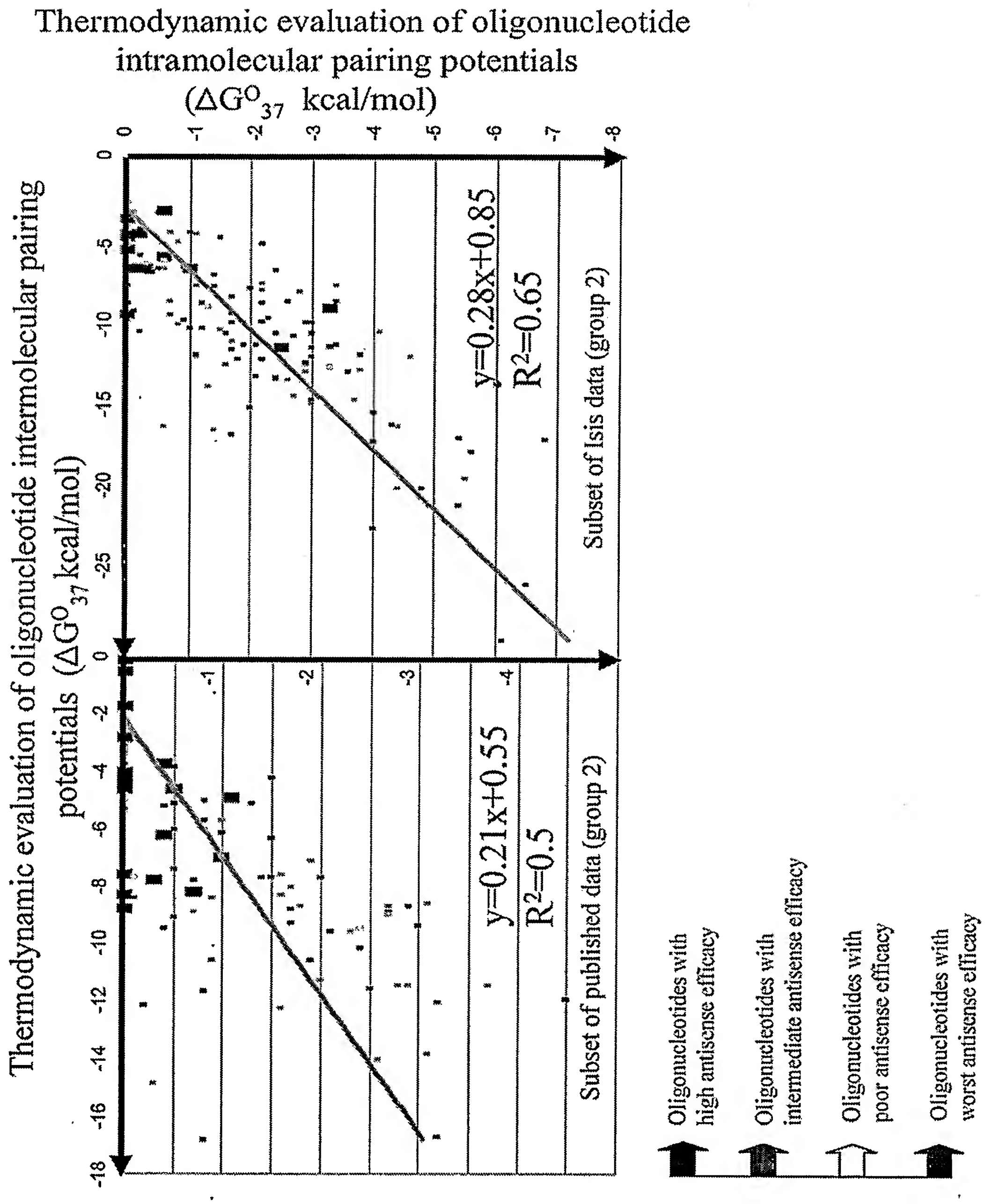

A
group 2
group 1
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
51
7
33
343
152
21
-40
-30
-10
Oligonucleotides with worst antisense efficacy
Oligonucleotides with poor antisense efficacy
Oligonucleotides with intermediate antisense efficacy
Oligonucleotides with high antisense efficacy
ΔG°37 (kcal/mol)
oligo-RNA duplex formation
B
10
18
2
8
4
7
11
-6.8
-1.1
-0.5
0
ΔG°37 (kcal/mol)
oligo intramolecular pairing
C
39
17
3
9
6
2
14
10
-29
-8
-5
0
ΔG°37 (kcal/mol)
oligo intermolecular pairing

Figure 11

Figure 12



Thermodynamic evaluation of oligonucleotide intramolecular pairing potentials (ΔG^{O}_{37} kcal/mol)
Thermodynamic evaluation of oligonucleotide intermolecular pairing potentials (ΔG^{O}_{37} kcal/mol)
y=0.28x+0.85
R^2=0.65
Subset of Isis data (group 2)
y=0.21x+0.55
R^2=0.5
Subset of published data (group 2)
Oligonucleotides with high antisense efficacy
Oligonucleotides with intermediate antisense efficacy
Oligonucleotides with poor antisense efficacy
Oligonucleotides with worst antisense efficacy

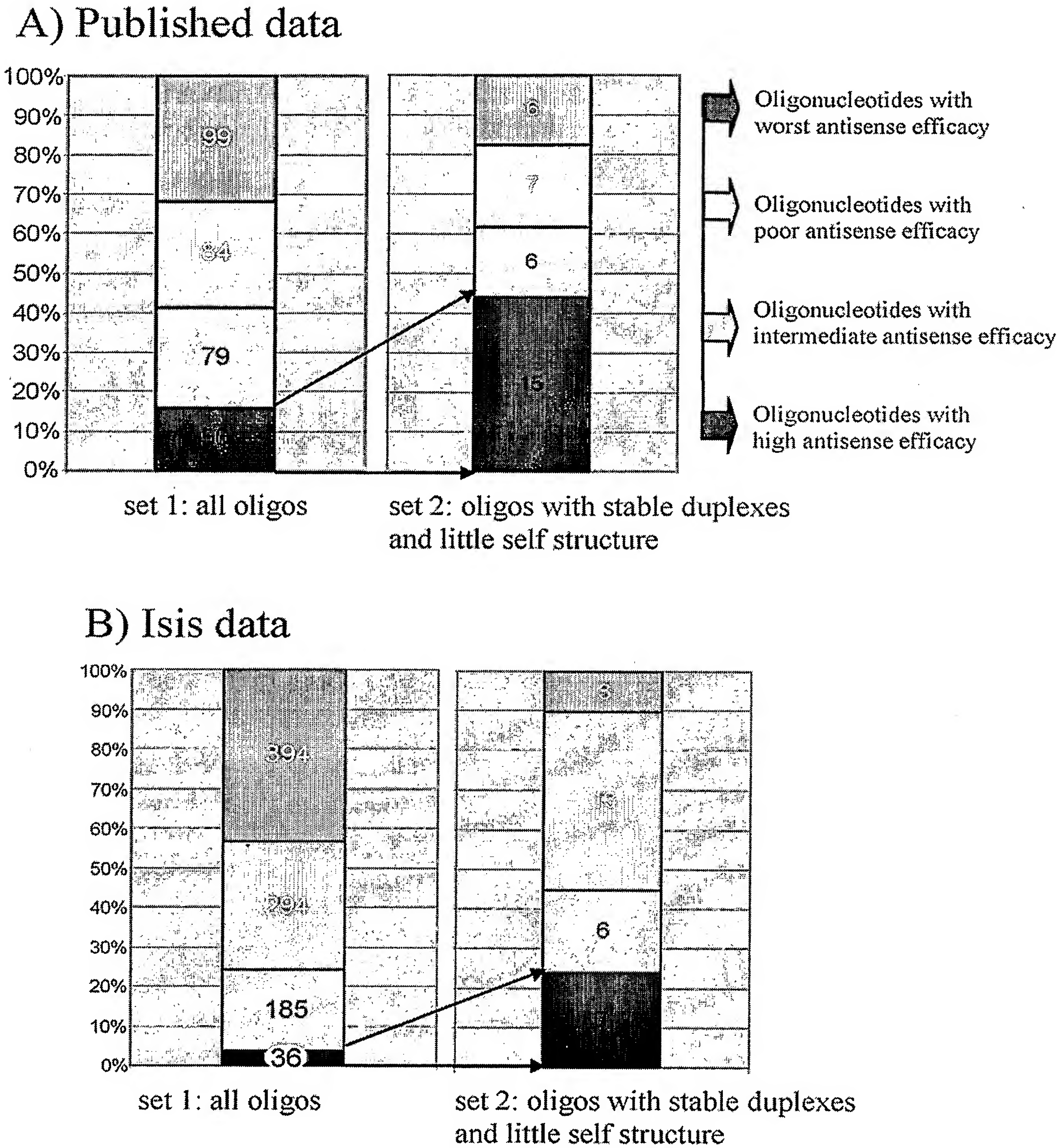

A) Published data
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
99
84
79
6
7
6
15
Oligonucleotides with worst antisense efficacy
Oligonucleotides with poor antisense efficacy
Oligonucleotides with intermediate antisense efficacy
Oligonucleotides with high antisense efficacy
set 1: all oligos
set 2: oligos with stable duplexes and little self structure
B) Isis data
394
294
185
36
3
6
set 1: all oligos
set 2: oligos with stable duplexes and little self structure

Figure 13

Figure 14A

```
790  ATGGGTGCGA GAGCGTCAGT ATTAAGCGGG GGAAAATTAG ATGCATGGGA AAAAATTCGG  849
850  TTAAGGCCAG GGCGAAAGAA AAAATATAGA CTAAAACATC TAGTATGGGC AAGCAGGGAG  909
910  CTGGAAAGAT TTGCACTTAA CCCTGGCCTT TTAGAAACAT CAGAAGGCTG TAAACAAATA  969
970  ATGGGACAGC TACAACCAGC TCTTCAGACA GGATCAGAAG AACTTAGATC ATTATATAAT 1029
1030 ACAGTAGCAA CCCTCTATTG TGTACATCAA AGGATAGAGG TAAAAGACAC CAAGGAAGCT 1089
1090 TTAGAGAAGA TAGAGGAAGA ACAAAACAAA AGTAAGCAAA AGACACAGCA GGCAGCAGCT 1149
1150 GACACAGGAA ACAGCAGCCA GGTCAGCCAA AATTACCCTA TAGTGCAGAA TCTACAAGGG 1209
1210 CAAATGGTAC ACCAGGCCAT ATCACCTAGA ACTTTGAATG CATGGGTAAA AGTAATAGAA 1269
1270 GAAAAGGCTT TCAGCCCAGA AGTAATACCC ATGTTTTCAG CATTATCAGA AGGAGCCACC 1329
1330 CCACAAGATT TAAACACCAT GCTAAACACA GTGGGGGGAC ATCAAGCAGC CATGCAAATG 1389
1390 TTAAAAGATA CCATCAATGA GGAAGCTGCA GAATGGGATA GGTTACATCC AGTACATGCA 1449
1450 GGGCCTATTC CACCAGGCCA GATGAGAGAA CCAAGGGGAA GTGACATAGC AGGAACTACT 1509
1510 AGTACCCTTC AGGAACAAAT AGGATGGATG ACAAGCAACC CACCTATCCC AGTGGGAGAA 1569
1570 ATCTATAAAA GATGGATAAT CCTGGGATTA AATAAAATAG TAAGAATGTA TAGCCCTGTC 1629
1630 AGCATTTTGG ACATAAGACA AGGGCCAAAA GAACCCTTTA GAGACTATGT AGACAGGTTC 1689
1690 TTTAAAACTC TAAGAGCTGA GCAAGCTACA CAGGATGTAA AAAATTGGAT GACAGAAACC 1749
1750 TTGTTGGTCC AAAATGCGAA CCCAGATTGT AAGACCATTT TAAAAGCATT AGGACCAGGG 1809
1810 GCTACACTAG AAGAAATGAT GACAGCATGT CAGGGAGTGG GAGGACCCAG CCATAAAGCA 1869
1870 AGAGTTTTGG CTGAGGCAAT GAGCCAAGCA ACAAATGCAG CCATAATGAT GCAGAGAGGC 1929
1930 AATTTTAAGG GCCAAAGAAG AATTATTAAG TGTTTCAACT GTGGCAAAGA AGGACACATA 1989
1990 GCCAGAAATT GCAGGGCCCC TAGGAAAAAG GGCTGTTGGA AATGTGGAAA GGAAGGACAC 2049
2050 CAAATGAAAG ACTGCACTGA AAGACAGGCT AATTTTTTAG GGAAAATTTG GCCTTCCAAC 2109
2110 AAGGGGAGGC CAGGGAATTT TCTTCAGAGC AGACCAGAGC CAACAGCCCC ACCAGCAGAG 2169
2170 AGCTTCGGGT TCGGGGAGGA GATAACCCCC TCTCCGAAGC AGGAGCAGAA AGACAAGGAA 2229
2230 CTGTATCCTC CTTTAGCTTC CCTCAAATCA CTCTTTGGCA ACGACCCCTT GTCACAATAA 2289
2290
```

Figure 14 B

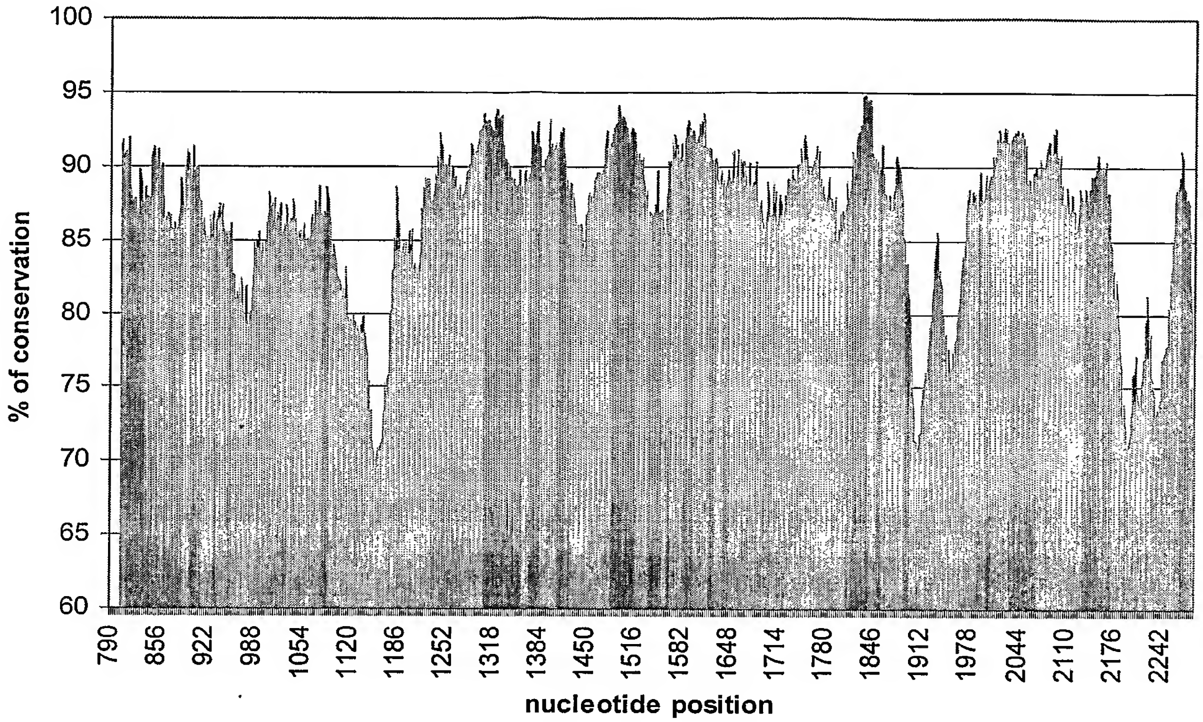
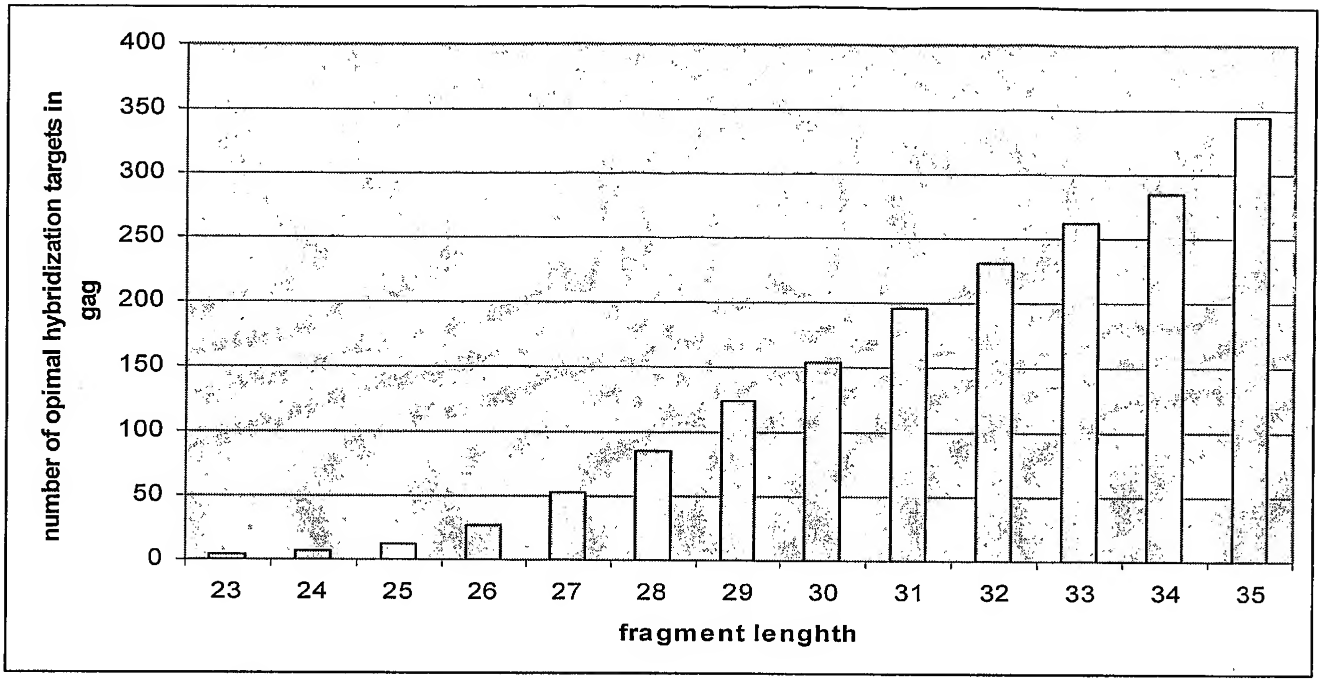

100
95
90
85
80
75
70
65
60
% of conservation
790
856
922
988
1054
1120
1186
1252
1318
1384
1450
1516
1582
1648
1714
1780
1846
1912
1978
2044
2110
2176
2242
nucleotide position

Figure 15



400
350
300
250
200
150
100
50
0
number of opimal hybridization targets in gag
23
24
25
26
27
28
29
30
31
32
33
34
35
fragment lenghth